Molecular Genetics and Otolaryngology

Michael E. Prater, MD
Shawn D. Newlands, MD
Introduction

- Chromosomal analysis
- Cytogenetics
- Molecular biology and genetics
- Biochemical genetics
- Clinical genetics
- Population genetics
- Genetic epidemiology
- Developmental genetics
- Immunogenetics
- Genetic counseling
- Fetal genetics
History

- **Gregor Mendel, 1865**
  - “Mendel’s Laws” of autosomal inheritance
  - Work “lost” until early 1900’s

- **Charles Darwin, 1859**
  - “The Origin of Species”
  - Jean Baptiste Lemarck
History, continued

- Francis Galton (Charles Darwin’s cousin)
  - The “father” of modern genetics
  - rediscovered Mendel’s laws
  - “nature versus nurture”
  - “inborn errors of metabolism” responsible for biological abnormalities
History, Continued

- James Watson and Francis Crick
  - DNA discovered in 1940’s
  - Determined double helix in 1953
  - Nobel Prize in 1962

- Human Genome Project
  - Begun in 1990
  - Goal is to identify every human gene by 2005
  - 9% completed as of 1999
Classification of Disorders

Single Gene Defects
- Usually single critical error in the genetic code
- Usually phenotypically obvious
- Examples: NF I and II, osteogenesis imperfecta, cystic fibrosis
Classification, continued

- Chromosomal disorders
  - not due to single defect
  - usually due to deficiency in number of genes within chromosome
  - classic example is Down Syndrome (Trisomy 21)
  - other examples: Trisomies 13, 18, Klinefelter’s Syndrome, Turner’s Syndrome
  - phenotypically obvious
  - usually incompatible with life
Classification, continued

- Multifactorial inheritance
  - multiple single code defects
  - usually form a pattern
  - classic examples: cleft lip/palate, neural tube defects
  - possible example: head and neck cancer?
Chromosomal Structure

- 23 pairs of chromosomes
- approximately 7 million base pairs
- 100,000 genes
- DNA:
  - five carbon sugar (deoxyribose; ribose in RNA)
  - nitrogen base (purines, pyrimidines)
  - 3’5’ phosphate linkage
  - hydrogen bonded double strand
DNA Bases

Purines
- Adenine (A)
- Guanine (G)

Pyrimidines
- Thymine (T)
- Cytosine (C)

Phosphate Deoxyribose

Base
DNA Bases
Transcription

The Central Dogma
Tools of Genetics

- Revolutionary changes since late 1970’s
  - restriction enzymes
  - recombinant DNA
  - vectors
  - probes
  - PCR
  - DNA sequence analysis
  - protein analysis
Tools of Genetics, cont.

- **Restriction Endonucleases**
  - enzymes which cleave DNA at specific sites
  - almost always palindromic
  - hundreds of known endonucleases

- **Recombinant DNA**
  - an DNA fragment is combined with a known piece of DNA to form a plasmid
  - plasmid inserted in vector (bacterium, virus, yeast)
  - vector cultured and isolated
Tools, continued

- Identification of recombinant fragments
  - “Blotting” - southern, northern, western
    - electrophoresis/chromotography of fragment
    - hybridization with known radioactive fragment
    - antibodies to known fragments may be used
Tools, continued

- Polymerase Chain Reaction (PCR)
  - simplest, most rapid, most effective
  - enzymatic amplification of desired fragment
  - DNA fragment formed by endonuclease
  - known “primer” is annealed to fragment
  - steps repeated approximately 30 times
  - yields more than a billion copies of desired DNA fragment
Tools, continued

- DNA Sequence Analysis
  - Fred Sanger, Nobel Prize 1980
    - also won Nobel Prize in 1958 for protein analysis
  - nucleotide analog with inhibits DNA synthesis
  - endonuclease which cleaves at nucleotide site
  - electrophoresis/chromotography
  - radioactive tagging/antibodies
Genetic Mutations

Defn: Permanent change in nucleotide sequence

- occur in somatic cells or germline cells
  - only germline cells inherited

- somatic mutations believed responsible for many medical problems
  - many cancers, ?CAD
Gentic Mutations, cont.

Genome Mutations

- missegregation of chromosome
  - results in aneuploidy
  - Down Syndrome classic example
  - 1:50 meiotic divisions
  - usually incompatible with life
Genetic Mutations, cont.

- **Chromosome mutations**
  - usually involve translocations and rearrangements
  - 1:1000 meiotic divisions
  - almost uniformly incompatible with life

- **Gene mutations (single gene defects)**
  - DNA replicates 20 bases/sec/polymerase
  - Only one defect per ten million copies
  - Repair enzymes repair 99.9% of defects
  - Less than one defect per 10 billion bases!
Genetics and Cancer

- Tumor cells are clone of abnormally dividing cell
  - usually from single/multiple point mutations
  - rarely from translocations

- Protooncogenes
  - normal growth genes

- Oncogenes
  - a protooncogene which has undergone somatic mutation and is oncogenic
Genetics/Cancer, cont.

- Tumor Suppressor Genes
  - genes that regulate cell growth/genomic expression
  - p53, Bcl-2 are classic examples
  - p53:
    - arrests growth in G1 (growth 1) phase
    - allows repair of DNA defects
    - induces apoptosis (programmed cell death)
    - found in 40% of HNSCCa
    - have NOT shown correlation with prognosis
Bcl-2 tumor suppressor gene

- normal Bcell lymphoma/leukemia gene (Bcl-2)
- prevents apoptosis (programmed cell death)
- somatic mutations present HNSCC, usually resulting in overexpression

Friedman’s study:
- retrospective study of Stage I/II HNSCCa
- overexpression of Bcl-2 lead to 50% cure versus 90% in normal expression
- others unable to reproduce (see Gallo)
Treatment

- Most disease treated at phenotypic level
  - medicines
  - surgery
  - genetic counseling

- Molecular level
  - gene therapy
Treatment, continued

Gene Therapy

- attempted modification of abnormal cell function
- involves transfer of functioning genes
- gene therapy via addition
  - more practical
  - insertion into cell (not necessarily into genome) of functioning gene
- gene therapy via replacement
  - theoretical
  - goal is to replace abnormal gene with inserted gene
Treatment, continued

Gene therapy, continued

Transfer strategies
- recombinant DNA in vector
  - viral versus bacterium
  - retroviral vectors with reverse transcriptase
- not inserted into host genome

Problems:
- inability to maintain expression
- under/overexpression
- adenine deaminase deficiency (ADA)
Genetic Disease in ENT

**Cystic Fibrosis**

- chromosome 7q, spans 250,000 bases
- 70% have deletion of phenylalanine at position 508 (point mutation)
  - frameshift versus point mutation
- most common fatal autosomal disease in whites
- phenotypic expression results from failure of membrane transport (Cl, Na) and from exocrine function (pancreas)
- Tx at phenotypic level
Genetic Dz in ENT, cont.

- Cleft Lip and Palate
  - one of the most common malformations
  - CL and P genetically distinct from isolated CL
  - failure of fusion of frontal process with maxillary process at 35 days gestation
  - classically described as multifactorial, although single gene forms, chromosomal forms (Trisomy 13) teratogenic forms (rubella, thalidomide) are known
Genetic Dz in ENT, cont.

- Human papilloma virus
  - strains 16, 18 and 31 carcinogenic in GU tract
  - exact role in HNSCCa not fully known, although 46% of post mortem specimens contained HPV strains
  - E6 HPV protein binds to p53 forming mutation which suppresses gene function in vivo
Genetic Dz in ENT, cont.

Thyroid carcinoma

- Medullary thyroid carcinoma (MTC)
  - neoplasm of parafollicular C cells (ultimobranchial body)
  - produce calcitonin
  - sporadic and familiar forms
  - familial MTC associated with MEN 2A and 2B
    - MEN 2A: pheo, hyperparathyroid, MTC
    - MEN 2B: pheo, MTC, Marfan’s, NFI
  - RET protooncogene associated with familial forms
    - 10p

- Aggressive papillary CA associated with aneuploidy
  - noninvasive dz uniformly diploid
Genetic Dz in ENT, cont.

Salivary Gland Neoplasms

- Aggressive adenoid cystic Ca associated with aneuploidy
  - all patients with aneuploidy recurred after resection versus only 2/14 with diploid genome (Sugano)
- Salivary gland adenocarcinoma with overexpression of Bcl-2 were more difficult to resect, recurred more frequently and metastasized more frequently (Sugano)
Genetic Dz in ENT, cont.

- Acoustic Neuroma
  - 5% are familial and associated with NF II
  - often bilateral
  - NF II defect on 22p
  - therapy at phenotypic level
Genetic Dz in ENT, cont.

**Congenital Hearing Loss**
- 60% of congenital hearing loss is genetic
- most associated with phenotypic anomaly
- Waardenburg Syndrome
  - autosomal dominant - variable penetrance
  - dystopia canthorum, hyperchromatic iris, white forelock and SNHL
  - PAX3 locus of chromosome 2
  - treatment at phenotypic level
Genetic Dz in ENT, cont.

- Congenital hearing loss, continued
  - Usher’s Syndrome
    - autosomal recessive
    - five different classifications (Usher’s Types I through V)
      - all subtypes on different chromosomes
    - associated with retinitis pigmentosum
    - therapy at phenotypic level
Genetic Dz in ENT, cont.

- Congenital Hearing Loss, continued
  - Pendred’s Syndrome
    - autosomal recessive with variable penetrance
    - located on chromosome 7q
    - associated with thyroid goiter and carcinoma
    - tx at phenotypic level
Genetic Dz in ENT, cont.

- Congenital hearing loss, cont.
  - Alport’s Syndrome
    - two forms: X linked, autosomal recessive
      - X linked on 5p, produces mutant alpha 5 protein
      - recessive form on 2p, produces mutant Type IV collagen
    - treatment at phenotypic level
Genetic Dz in ENT, cont.

- Head and Neck Cancer
  - heavily associated with p53 underexpression, Bcl-2 overexpression, HPV types 16, 18 and 31
  - None of these proven prognostic
  - Ultimate goal: gene therapy to correct somatic mutation
Future Directions and Conclusion

- Rapidly expanding field
- Ultimate goal: correction of somatic defect which would correct phenotypic abnormality. Would eliminate surgical intervention.